

**RADIOSENSITIZING TAXANES AND
THEIR PHARMACEUTICAL PREPARATIONS**

This application is a continuation of 09/723,719 filed 11/28/2000, now abandoned, which is a continuation of 09/129,647 filed 09/05/1998, now abandoned, which is a continuation of 08/710,240 filed 09/13/1996, now abandoned, which claims benefit of provisional application 60/003,687, filed on 09/13/1995.

Background of the Invention

This invention relates to novel
5 radiosensitizing compounds, and in particular, to substituted taxanes containing radiosensitizing moieties, their pharmaceutical preparations, and methods of using this new class of highly potent radiosensitizers of tumor cells.

10 In the United States alone, over a half million patients undergo radiation therapy each year as a part of their battle against cancer. To date, however, radiation therapy has produced only limited success as a cancer treatment. Understandably, therefore, a major effort has
15 been underway for a number of years to develop means to improve the efficacy of such radiotherapy techniques.

It is widely believed that the presence of radioresistant, hypoxic (poorly oxygenated) cells in tumors constitutes a significant factor in causing local
20 failure in conventional cancer radiotherapy. For example, it was reported by Gatenby et al., Int. J. Radiat. Oncol. Biol. Phys. 14: 831-833 (1988), that for head and neck tumors, the hypoxic cell volume is inversely correlated with tumor radiosensitivity. Other
25 reports confirm this conclusion for a variety of types of tumors and suggest that the presence of a concentration of as little as 2-3% hypoxic cells in a tumor may double the radiation dose required for tumor control.

Various solutions have been proposed to
30 overcome the problem of hypoxia, including carrying out radiation treatments in high pressure oxygen chambers and the substitution of "fast neutron" or π meson radiation in place of X-rays. However, these techniques are not wholly satisfactory for a number of reasons, including

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the great expense and difficulty frequently associated with such procedures.

One promising field of investigation for dealing with radioresistant hypoxic tumor cells has been the use of "radiosensitizing" compounds which selectively increase the sensitivity of hypoxic cells to radiation. This specificity to hypoxic cells is also valuable because a significant percentage of solid tumors are characterized by such cells while most normal tissue is not. Thus, treatment with such compounds serves to enhance the impact of radiation on tumor cells while having little effect on the impact of radiation on healthy cell tissue. A number of heterocyclic, electron-affinic compounds, and in particular, those with oxidized nitrogen moieties, have been successfully used for the purpose of radiosensitizing hypoxic tumor cells. Specifically, the discovery that the nitroimidazoles, metronidazole (metro) and misonidazole (miso), sensitize hypoxic cells to radiation provided initial optimism for a breakthrough solution to the problem of tumor hypoxia. Unfortunately, however, both agents have proven to be highly toxic at therapeutic levels.

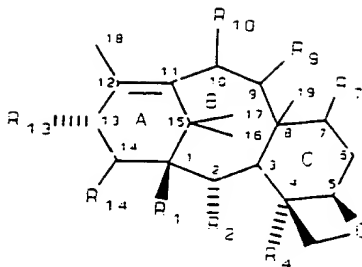
The possibility of using chemotherapeutic agents to selectively enhance radiation response in tumors has also been proposed. In addition to its use as a chemotherapeutic agent, taxol, for example, has been investigated in vitro and in vivo as a potential radiosensitizing drug. See, Tishler et al., Radiation Oncology Biol. Phys., 22:613-617 (1992); Tishler et al., Cancer Research, 52:3495-3497 (1992); Steren, et al., Gynecologic Oncology, 48:252-258 (1993); Steren, et al., Gynecologic Oncology, 50:89-93 (1993); Choy et al., Cancer, 71:3774-3778 (1993); Milas et al., Cancer Research, 54:3506-3510 (1994); and Joschko et al., Proceedings of the American Association for Cancer

Research, 35:647 (1994). Although the reported data suggests that taxol is an effective radiosensitizer, recent data from our laboratory leads us to question whether the reported data has been misinterpreted. In
5 any event, a need continues to exist for compounds which possess antitumor activity and which are more potent radiosensitizers and thus, can be administered at lower doses to reduce toxic side effects.

Summary of the Invention

10 Among the several objects of the invention, therefore, may be noted the provision of a novel class of compounds and pharmaceutical preparations containing them which possess antitumor activity and which are potent radiosensitizing agents for cancer radiation therapy.
15 Also among the objects of the invention are methods for the use of such compounds and pharmaceutical preparations in warm-blooded animals in radiation therapy.

Briefly, therefore, the present invention is directed to taxanes comprising one or more electron-affinic moieties. Such compounds provide greatly
20 enhanced radiosensitization of tumors and reduced toxic side effects to normal body tissues at a given dosage as compared to conventional radiosensitization agents. The electron-affinic moiety may be attached directly, or
25 indirectly through a linker to one of the ring atoms of the taxane or to one of the C13 side chain atoms. For example, the electron-affinic moiety may be attached to the C2, C4, C7, C9, C10, C14, C3' or C5' carbon of a taxane corresponding to the structure:



wherein

M comprises ammonium or is a metal;

R_1 is hydrogen or hydroxy;

R_2 is $-OT_2$, $-OCOZ_2$, $-OCOOZ_2$, RSG_1 or RSG_2 ;

R_4 is $-OT_4$, $-OCOZ_4$, $-OCOOZ_4$, RSG_1 or RSG_2 ;

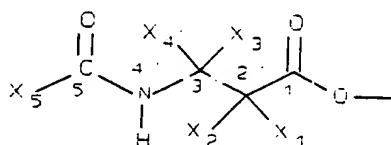
R_7 is hydrogen, halogen, $-OT_7$, $-OCOZ_7$, $-OCOOZ_7$, RSG_1 or RSG_2 ;

R_9 is hydrogen, keto, $-OT_9$, $-OCOZ_9$, $-OCOOZ_9$, RSG_1 or RSG_2 ;

R_{10} is hydrogen, keto, $-OT_{10}$, $-OCOZ_{10}$, $-OCOOZ_{10}$, RSG_1 or RSG_2 ;

R_7 , R_9 , and R_{10} independently have the alpha or beta stereochemical configuration;

R_{13} is hydroxy, protected hydroxy, keto, MO- or



R_{14} is hydrogen, hydroxy, protected hydroxy, RSG_1 or RSG_2 ;

T_2 , T_4 , T_7 , T_9 and T_{10} are independently hydrogen or hydroxy protecting group;

X_1 is $-OX_6$;

X_2 is hydrogen, hydrocarbon, heterosubstituted hydrocarbon, heteroaryl or heterosubstituted heteroaryl;

X_3 and X_4 are independently hydrogen, hydrocarbon, heterosubstituted hydrocarbon, heteroaryl, heterosubstituted heteroaryl or RSG_1 ;

X_5 is $-X_{10}$, $-OX_{10}$, $-SX_{10}$, or $-NX_8X_{10}$;

5 X_6 is hydrogen, hydrocarbon, heterosubstituted hydrocarbon, heteroaryl, heterosubstituted heteroaryl or hydroxy protecting group or a functional group which increases the water solubility of the taxane derivative;

10 X_8 is hydrogen, hydrocarbon, heterosubstituted hydrocarbon, RSG_1 or RSG_2 ;

X_{10} is hydrocarbon, heterosubstituted hydrocarbon, heteroaryl, heterosubstituted heteroaryl, RSG_1 or RSG_2 ;

15 Z_2 , Z_4 , Z_7 , Z_9 and Z_{10} are independently hydrocarbon, heterosubstituted hydrocarbon, heteroaryl or heterosubstituted heteroaryl;

RSG_1 is an electron-affinic moiety;

RSG_2 is $-L-(RSG_1)_n$;

20 L is a linker comprising a chain of 1 to 30 atoms in the chain, the atoms being selected from the group consisting of C, O, N, S, Si, and P; and

n is an integer greater than or equal to 1.

The invention is also directed to pharmaceutical compositions for radiosensitizing tumor
25 cells which contain a radiosensitizing amount of the above described taxanes or a pharmaceutically acceptable salt thereof in admixture with a pharmaceutically acceptable carrier.

30 The present invention is further directed to a process for radiosensitizing tumor cells. The process comprises administering a radiosensitizing amount of the pharmaceutical composition described above to the tumor cells. Related thereto, a method is also provided for
35 killing tumor cells in a warm-blooded animal which includes the steps of administering to the warm-blooded

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animal a pharmaceutical composition as described above in an amount effective to radiosensitize the tumor cells, followed by, after a time interval sufficient to enhance radiosensitization of the tumor cells, irradiating the tumor cells with a dose of radiation effective to kill the tumor cells.

Other objects and features will be in part apparent and in part pointed out hereinafter.

Brief Description of the Drawings

Fig. 1 is a graph depicting in vitro chemotherapeutic activity of taxoltere metro, taxol and taxoltere pnip on CHO cells for the studies set forth in Example 4.1.

Fig. 2 is a graph depicting in vitro chemotherapeutic activity of taxoltere, taxol and taxoltere pnip on HCT 116 cells for the studies set forth in Example 4.1.

Fig. 3 is a graph depicting in vitro chemotherapeutic radiosensitization of taxoltere metro and taxoltere pnip on CHO cells for the studies set forth in Example 4.2.

Fig. 4 is a graph depicting in vitro chemotherapeutic radiosensitization of taxoltere metro and taxoltere pnip on HCT 116 cells for the studies set forth in Example 4.2.

Fig. 5 is a graph depicting in vivo dose-response curves for taxoltere metro and taxol for the studies set forth in Example 4.4.

Fig. 6 is a graph depicting in vivo chemotherapeutic activity of taxoltere metro and taxoltere pnip at 40% of LD₅₀ for the studies set forth in Example 4.4.

Fig. 7 is a graph depicting the chemotherapeutic effect of low-dose multi-treatment (Q7D

x 4) with taxol and its analogs on MTG-B mammary tumors for the studies set forth in Example 4.4.

Fig. 8 is a graph depicting the chemotherapeutic effects of low-dose multi-treatment (Q7D x 4) with taxol and its analogs on MTG-B mammary tumors for the studies set forth in Example 4.4.

Figs. 9 and 10 are graphs depicting the chemotherapeutic effects of low-dose multi-treatments (Q11D x 4) with taxol and its analogs on MTG-B mammary tumors for the studies set forth in Example 4.4.

Fig. 11 is a graph depicting in vivo chemotherapeutic radiosensitization of taxoltere metro, taxoltere pnip and taxol on MTG-B mammary tumors (i.p., single dose) for the studies set forth in Example 4.5.

Fig. 12 is a graph depicting the effects of ip taxol, taxoltere metro and taxoltere pnip +/-RT on MTG-B mammary tumors for the studies set forth in Example 4.5.

Figs. 13 and 14 are graphs depicting the effects of taxoltere pnip on MTG-B mammary tumors (i.v., single dose, 24% LD50) for the studies set forth in Example 4.5.

Fig. 15 is a graph depicting the cure rate for taxoltere pnip (i.p., single dose) for the studies set forth in Example 4.6.

Figs. 16 and 17 are graphs depicting the cure rate for taxoltere pnip (i.v., single dose) for the studies set forth in Example 4.6.

Fig. 18 is a graph depicting the cure rate for taxoltere pnip +/- RT on MTG-B mammary tumors in vivo as a function of drug dose for the studies set forth in Example 4.6.

Detailed Description of the Preferred Embodiments

Surprisingly, it has been discovered that taxanes containing electron-affinic substituents exhibit

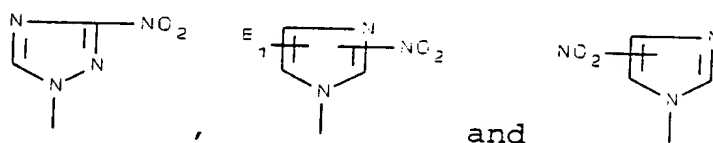
significantly greater potency than taxol as a radiosensitizing agent. As a result, such increased potency permits the administration of much lower dosages of these compounds for the same or even greater radiosensitization of tumor cells, allowing for a concomitant reduction in toxic side effects on healthy tissue for any particular dosage level required to effectively radiosensitize the tumor cells.

The radiosensitizing groups or moieties described herein (RSG₁ and RSG₂) impart electron affinity to the compounds with which they are associated. This novel class of potent radiosensitizers comprise taxanes containing at least one, and optionally two or more electron-affinic moieties. In general, the radiosensitizing moieties contain electron-affinic groups which fall into one of four groups: (i) carbocyclic or heterocyclic aromatic moieties which possess one or more carbonyl, trifluoromethyl, halogen, nitro, sulfonyl, sulfinyl, phosphoryl, oxide or cyano groups, (ii) heterocyclic aromatic moieties containing two or more heteroatoms, (iii) metal complexes, and (iv) organo-metallic groups in which the metal is covalently bonded to carbon.

The carbocyclic or heterocyclic aromatic electron-affinic moieties contain one to three rings with a total of 5 to 15 ring atoms which are selected from the group consisting of C, N, S, O and P. Preferably, the carbocyclic or heterocyclic aromatic electron-affinic moieties contain one to two rings with one ring being presently most preferred. Representative carbocyclic aromatic electron-affinic moieties include phenyl and naphthyl groups containing one or more nitro, halogen, carbonyl or sulfonyl substituents, with nitro-substituted phenyl being a preferred carbocyclic aromatic electron-affinic moiety. Representative heterocyclic aromatic

electron-affinic moieties include imidazoles, triazoles, pyridines, benzamides, nicotinamides, benzotriazine oxides, furans, thiophenes, oxazoles and thiozoles possessing one or more carbonyl, trifluoromethyl, halogen, nitro, sulfonyl, sulfinyl, phosphoryl, oxide or cyano groups, and preferably at least one nitro group.

Nitroimidazole and nitrotriazole heterocyclic aromatic electron-affinic moieties which may be incorporated into the radiosensitizing agents of the present invention include 2-nitroimidazol-1-yl and 3-nitro-1,2,4-triazol-1-yl and other nitroimidazoles and nitrotriazoles which correspond to the following structures:



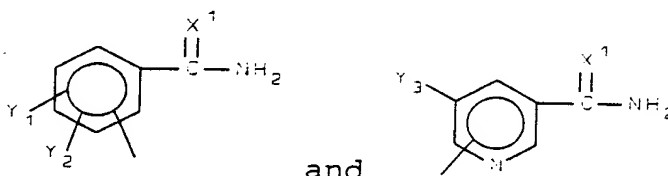
wherein E_1 is alkyl or fluoroalkyl. The preparation and use of radiosensitizing agents incorporating these and other nitroimidazoles and nitrotriazoles is described in Suzuki et al., U.S. Patent Nos. 4,945,102 and 5,064,849; Kagiza et al., U.S. Patent Nos. 4,927,941, 4,977,273 and 5,304,654; Suto, U.S. Patent No. 4,954,515 and 5,036,096; Suto et al., U.S. Patent No. 4,797,397; Papadopoulou-Rosenzweig et al., U.S. Patent No. 5,294,715; Beylin et al., U.S. Patent No. 5,342,959.

Benzamide and nicotinamide heterocyclic aromatic electron-affinic moieties which may be incorporated into the radiosensitizing agents of the present invention include

- 5-hydroxynicotinamide;
- 5-nitronicotinamide;
- 5-(2,3-dihydroxypropoxy)nicotinamide;

- 5-aminonicotinamide;
 5-(2-methoxyethylamino)nicotinamide;
 5-acetamidonicotinamide;
 3-hydroxy thiobenzamide;
 3-[(2-hydroxyethoxy)acetamido]benzamide;
 3-(2,3 dihydroxy-n-propoxy)-4-methoxybenzamide;
 3-(2,3 dihydroxy-n-propoxy)-4-methylbenzamide;
 4-(2,3 dihydroxy-n-propoxy)-3-methoxybenzamide;

and other benzamides and nicotinamides which correspond to the following structures:



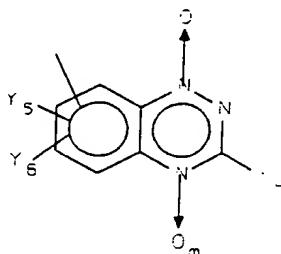
wherein X^1 is O or S; Y_1 is H, lower alkyl, lower alkoxy, acetoxy, or acetamido; Y_2 is -OR, -SR, -NHR, -NO₂, -O(CO)R, -NH(CO)R, -O(SO)R, or -O(POR)R; Y_3 is H, Z_1 , -OR, -SR, -NHR, -O(CO)R, -NH(CO)R, -O(SO)R, or -O(POR)R; and R is hydrogen or hydrocarbon which may be optionally substituted and interrupted by an ether (-O-) linkage. The preparation and use of radiosensitizing agents incorporating these and other benzamides and nicotinamides is described in Lee et al., U.S. Patent Nos. 5,032,617, 5,041,653 and 5,175,287.

Benzotriazine oxide heterocyclic aromatic electron-affinic moieties which may be incorporated into the radiosensitizing agents of the present invention include

- 3-hydroxy-1,2,4-benzotriazine-1,4-dioxide;
 3-amino-7-trifluoro-1,2,4-benzotriazine-1-oxide;
 3-amino-7-decyl-1,2,4-benzotriazine-1-oxide;

3-amino-7-carbamyl-1,2,4-benzotriazine-1-oxide;
7-acetyl-3-amino-1,2,4-benzotriazine-1-oxide;
7-chloro-3-hydroxy-1,2,4-benzotriazine-1,4-
dioxide;

5 7-nitro-3-amino-1,2,4-benzotriazine-1,4-
dioxide; and other benzotriazine oxides corresponding to
the structure:

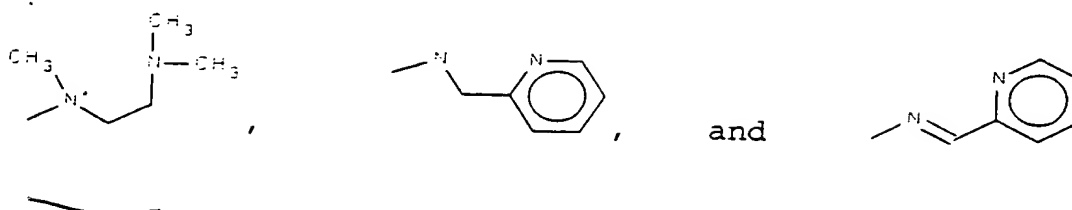


wherein Y_4 is H, substituted or unsubstituted lower
10 hydrocarbon, or alkanoyl; m is 0 or 1; and Y_5 and Y_6 are
independently hydrogen, nitro, halogen, morpholino,
pyrrolidino, piperidino, substituted or unsubstituted
hydrocarbon, $-NH_2$, $-NHR'$, $-NR'R'O(CO)R'$, $-NH(CO)R'$,
15 $-O(SO)R'$, or $-O(POR')R'$ in which R' is substituted or
unsubstituted hydrocarbon. The preparation and use of
radiosensitizing agents incorporating these and other
benzotriazine oxides is described in Lee et al. U.S.
Patent No. 5,175,287.

The metal complex electron-affinic moieties
20 preferably comprise Pt^{2+} , Co^{3+} , Fe^{2+} , Fe^{3+} , Pd^{2+} , Cu^{2+} , Ti^{4+} ,
or Zr^{4+} as the metal and generally fall into two
subgroups: (a) metal complexes of the carbocyclic and
heterocyclic aromatic electron-affinic moieties discussed
above, and (b) metal complexes of bidentate ligands
25 comprising nitrogen, carbon or sulfur. In general, metal
complexes of bidentate ligands correspond to the formula
 $-BM^LX_k$ wherein B is a bidentate ligand containing
nitrogen, carbon or sulfur, M^L is a metal, X is an anionic

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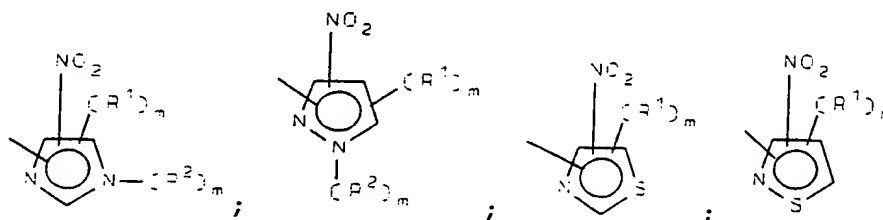
ligand such as Cl^- or OAc , and k is 1-4. Exemplary bidentate ligands include:



Electron-affinic metal complexes which may be incorporated into the radiosensitizing agents of the present invention include compounds of the formula:

$$[\text{PtX}^{\text{M}}_j(\text{NR}_2^{\text{H}}\text{Q})] \text{ or } [\text{PtX}^{\text{M}}_j(\text{NR}_2^{\text{H}})_2\text{Q}]^+\text{Y}^-$$

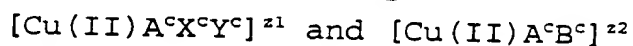
wherein n is 1 or 2, and wherein when n is 2, X^{M} is a monovalent biologically acceptable anion, and when j is 1, X^{M} is a divalent biologically acceptable anion; each R^{H} is independently H or alkyl, or both R^{H} 's together are a piperidino or morpholino residue; Q is a radiosensitizing ligand selected from a mononitro-substituted imidazole, a mononitro-substituted pyrazole, a mononitro-substituted thiazole and a mononitro-substituted isothiazole; and Y^- is a physiologically acceptable anion. These heterocycles may optionally be substituted by an alkyl, amino substituted alkyl, hydroxy, alkoxy or amino group. In addition, if the heterocycle is pyrazole or imidazole, a ring nitrogen may be substituted by alkyl or alkoxy or hydroxy substituted alkyl and wherein one or two methylenes of the alkyl may be replaced by oxygen. In a preferred embodiment, Q is one of the following:



wherein R^1 is alkyl optionally containing an amino substituent, $-OR^3$, or $-N(R^3)_2$, wherein R^3 is H or lower alkyl; R^2 is alkyl or 1-8 carbons substituted by one or more $-OR^3$ and wherein one or two methylenes may be replaced by oxygen and each m is independently 0 or 1. The preparation and use of radiosensitizing agents incorporating these metal complexes is described in Skov et al. U.S. Patent Nos. 4,921,963 and 5,026,694.

Other electron-affinic metal complexes which may be incorporated into the radiosensitizing agents of the present invention may be made by reacting an organic or inorganic platinum compound such as an alkali metal tetrahaloplatinate or cis-bis(acetonitrile)dichloroplatinum (II) with rhodamine 123 or other (+)-charged rhodamine or the like, for example, a cyanine dye such as 3,3'-diethylthiadibocyanine iodide or other (+)-charged cyanine dyes as described in U.S. Patent No. 5,196,413.

Other electron-affinic metal complexes which may be incorporated into the radiosensitizing agents of the present invention include include Cu(II) compounds selected from compounds having the formula:

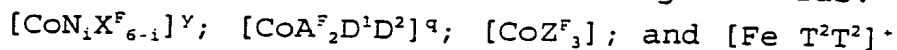


wherein A^c represents a bidentate heteroaromatic ligand containing neutral nitrogen donor atoms; B^c represents a bidentate ligand containing neutral or negatively charged oxygen donor atoms; X^c and Y^c are the same or different neutral or negatively charged monodentate ligands; and Z^1 and Z^2 represent the charge on the complex. The preparation and use of radiosensitizing agents incorporating these metal complexes is described in Abrams et al. U.S. Patent No. 5,100,885.

Other electron-affinic metal complexes which may be incorporated into the radiosensitizing agents

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include Co(III) or Fe(III) compounds a formula corresponding to one of the following formulas:



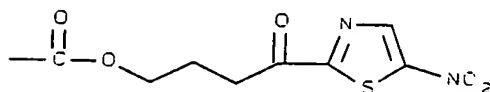
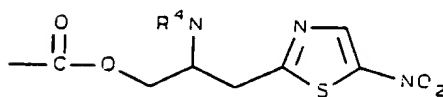
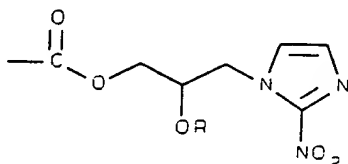
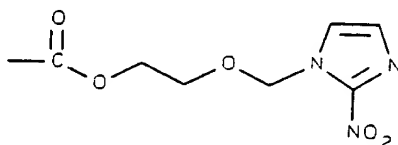
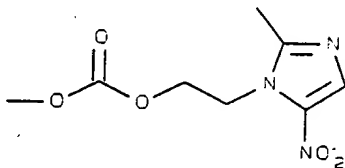
wherein n has a value of 3 or 4; N is an uncharged
5 nitrogen donor atom that is contained within a ligand; X^F
represents an anionic ligand; and y represents the charge
on the complex; A^F represents a bidentate or tetradentate
negative ligand containing N or O donor atoms; D^1 and D^2
10 represent the same or different monodentate ligands; q
represents a positive or negative charge on the complex;
 Z^F represents a chelating mononegative negative ligand; T^1
and T^2 , which may be the same or different, represent
mono-negative tridentate ligands. The preparation and
use of radiosensitizing agents incorporating these metal
15 complexes is described in U.S. Patent No. 4,727,068.

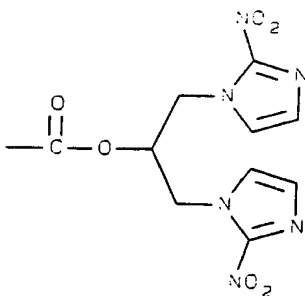
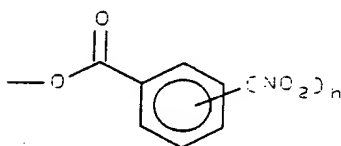
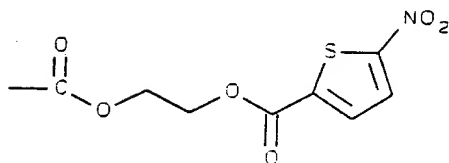
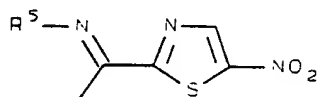
The organometallic electron-affinic moieties
are aliphatic or aromatic mercury radicals. The
preparation and use of radiosensitizing agents
incorporating mercury containing entities is described in
20 Shenoy et al., Cancer Investigation, 10(6):533-551 (1992)
and Bruce et al., Radiation Res., 24:473-481 (1965).

The electron-affinic moieties may be directly
attached to one of the carbons of the A, B, or C rings of
the taxane or indirectly attached via a linker. The
25 linker comprises a chain of 0 to 30 atoms in the chain,
with approximately 10 or less being preferred. The chain
atoms are selected from the group consisting of C, O, N,
S, Si, and P and are preferably C, N or O. The linker
may be linear or cyclic, branched or unbranched, and may
30 contain as substituents, one or more P, C, O, N, S, H, Si
or halogen-containing substituents. Exemplary linker
substituents include silyls, ethers, thioethers, esters,
thioesters, amides, thioamides, amines, alcohol, alkyl,
aryl, carbonyl, sulfonyl, phosphoryl, and halogen
35 substituents.

Preferably, the linker comprises a hydrocarbon segment consisting of 1 to 6 carbon atoms. It may additionally comprise a carbonyl, ester, thioester, amide, carbonate, thiocarbonate, carbamate, or ether segment. If a non-hydrocarbon segment is included; the non-hydrocarbon segment preferably comprises one or more ether, carbonate or carbonyl moieties as the non-hydrocarbon segment.

For purposes of illustration, a series of radicals comprising linkers and electronic-affinic moieties falling within the scope of the present invention is set forth as follows:

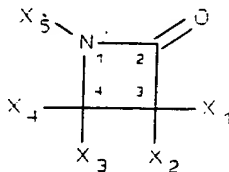




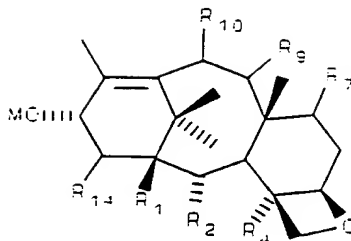
wherein h is 1-3, R⁴ is H, hydrocarbon or substituted hydrocarbon, and R⁵ is hydrocarbon or substituted hydrocarbon. In other embodiments, the carbonyl or ester linkage of the above structures may be replaced by thioester or amide linkages. In addition, many of these radicals may serve as ligands for the previously identified metal species.

The radiosensitizing compounds of the present invention are prepared by linking the electron-affinic moiety to the C2, C4, C7, C9, C10, C14, C3', or C5' carbons of a taxane. The starting material may be 10-deacetyl baccatin III, baccatin III, or another naturally occurring taxane such as 14-hydroxy-10-deacetyl baccatin III. Alternatively, the taxane may be synthesized from commodity chemicals as set forth in PCT Patent Application No. WO 95/03265.

Taxanes having C13 side chains which incorporate electron-affinic moieties at C3' and/or C5' may be prepared through the use of β -lactams having the desired substituents and reacting the β -lactam and a C13 metal or ammonium alkoxide of a suitably substituted taxane as more fully described in U.S. Patent 5,430,160. The β -lactams have the following structural formula:



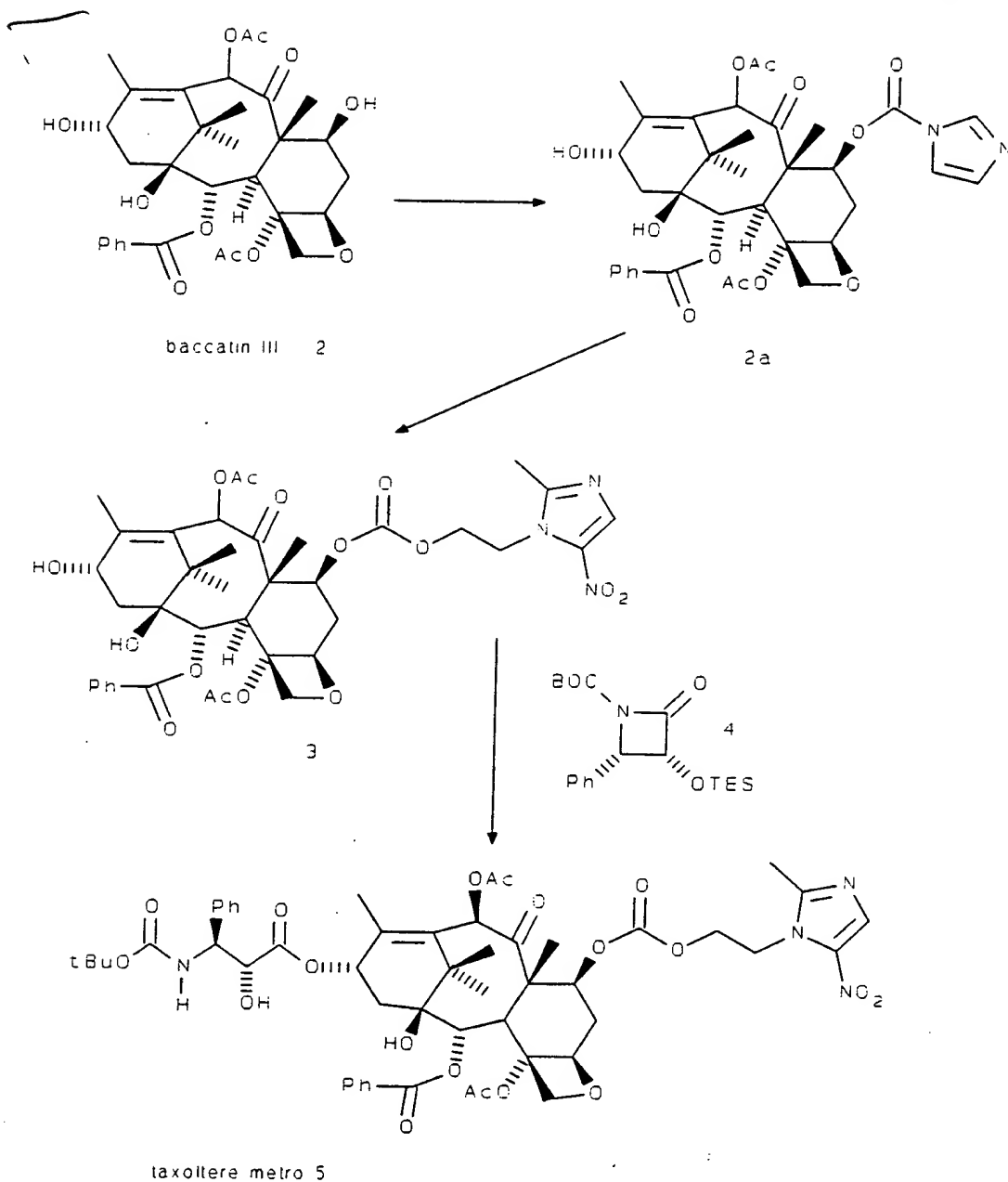
wherein X₁ is protected hydroxy, and X₂ - X₅ are as previously defined. Preferably, the alkoxide has the tetracyclic taxane nucleus and corresponds to the structural formula:



wherein M is a metal or tetraalkylammonium and R₁, R₂, R₄, R₇, R₉, R₁₀, and R₁₄ are as previously defined.

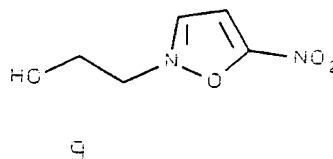
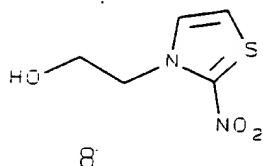
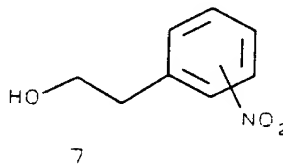
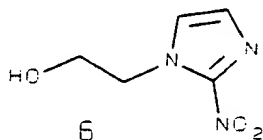
The electron-affinic moieties can be attached to the C2, C4, C7, C9, C10, and C14 positions of a taxane by a variety of methods. For purposes of illustration, the attachment method will first be described with respect to the C7 position. As will be described elsewhere herein, however, these same methods can be used for the other positions.

Metronidazole, a well known radiosensitizer, and other electron-affinic moieties can be attached via a carbonate linkage by treating baccatin III 2 with carbonyl diimidazole to produce the 7-carbonylimidazolide 2a, and reacting the product in situ with metronidazole at higher temperature to provide the 7-carboxylmetronidazolide 3. A C13 side chain can then be attached to 7-carboxylmetronidazolide 3 by treating it with lithium hexamethyldisilazide and β -lactam 4, and, after treatment with HF, to yield a taxane which we have named taxoltere metro 5. This reaction sequence is summarized in the following reaction scheme:



This method can be used for the preparation of a series of similar radiosensitizing taxanes having different radiosensitizing groups. The intermediate 7-carbonylimidazolidine 2a reacts smoothly with alcohols to provide the desired radiosensitizing taxanes in which the radiosensitizing group is attached via a carbonate linkage, as in taxoltere metro. For example, alcohols 6

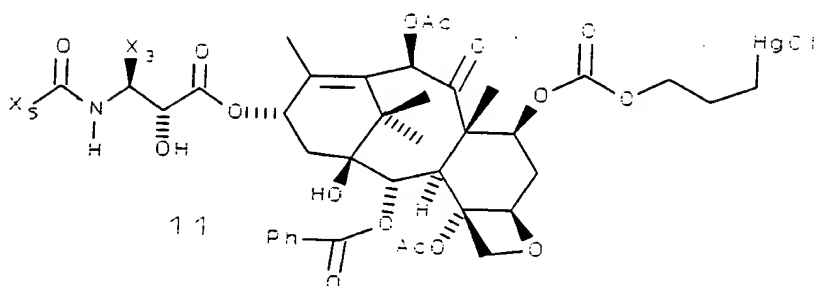
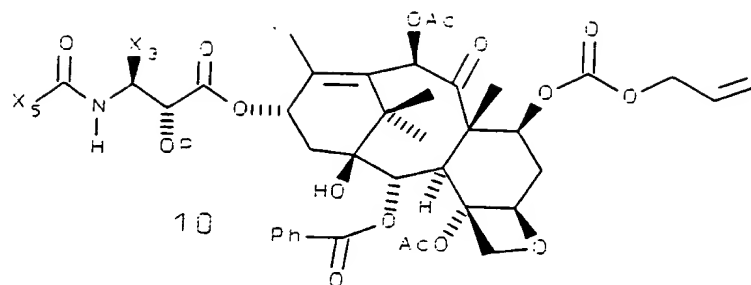
through 9 will react with the carbonylimidazolidine substituent of 2a to yield four other taxanes having radiosensitizing groups linked to C7.



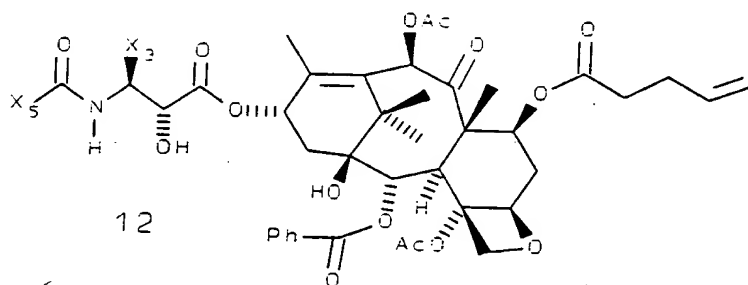
- 5 Attachment of the radiosensitizing group is then followed by attachment of a side chain at C13 in the same manner as it was accomplished for taxoltere metro.

- 10 The attachment of a metal atom or metal complex tethered at the C7 position of the taxane core can be accomplished by reacting an allylchloroformate with a taxane having an available C7 hydroxy group and a protected 2' hydroxy group to produce derivative 10 wherein X₃ and X₅ are as previously defined and P is a hydroxy protecting group.
- 15 carbonate substituent followed by treatment of the borane with mercuric acetate and sodium chloride and deprotection of the C2' hydroxy group gives the mercury derivative 11, a good radiosensitizer.

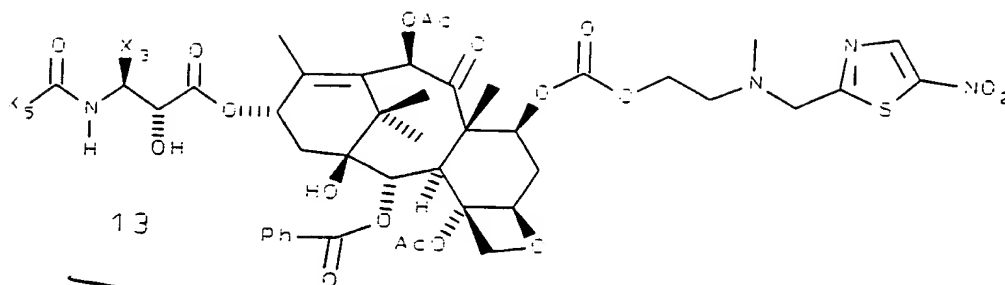
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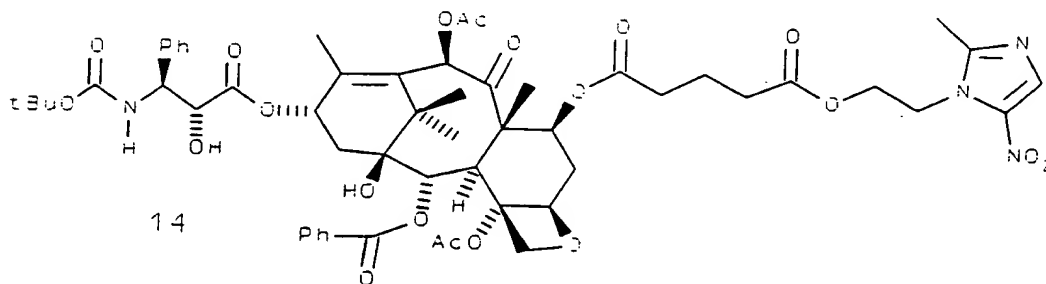
The ester analog of 11 can be prepared by the direct acylation of the C7 hydroxyl group of a 2' hydroxy protected taxane with an acid chloride to produce ester 12. Hydroboration of the allyl ester followed by treatment of the borane with mercuric acetate and sodium chloride gives the ester analog of mercury derivative 11.

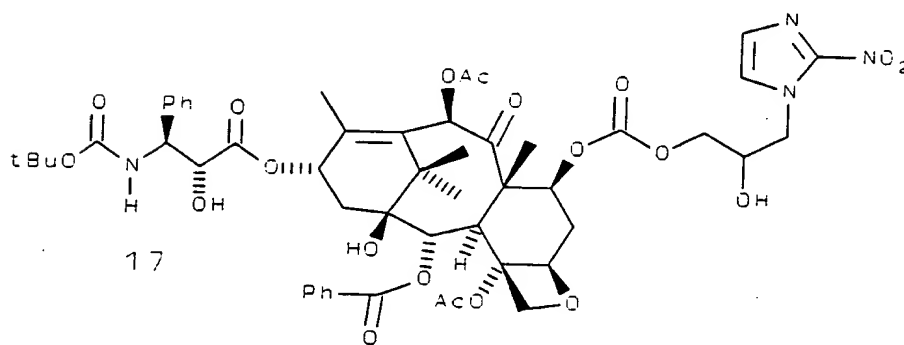
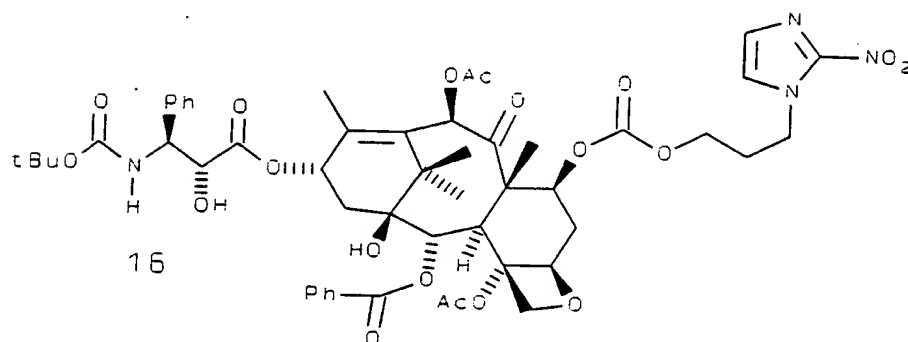
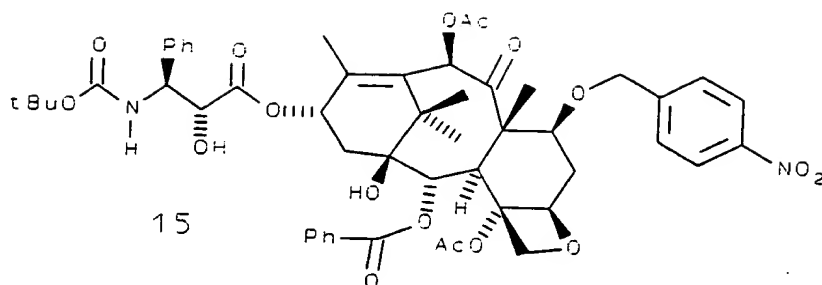


Similar chemistry can be used to attach a bidentate ligand to the C7 position, and the metal complex (e.g., platinum) of the bidentate ligand can then be prepared by introducing an appropriate metallic reactant (e.g., PtCl₂ or PtCl₂(SMe₂)₂). The bidentate ligand can also incorporate an electron-affinic ligand, and the Pt (II) complex of 13 can be made in this way.

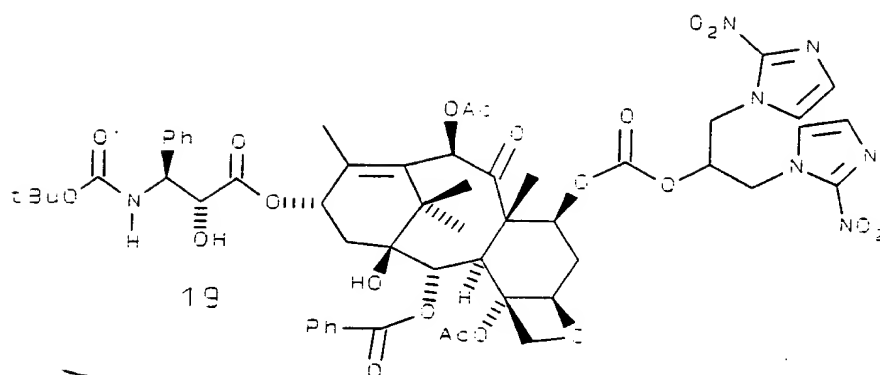
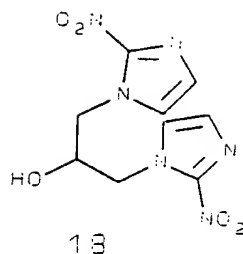


The length and nature of the linker between the taxane and the electron-affinic group may be altered. It is possible to prepare analogs in which the electron-affinic group is both closer and further away from the taxane than it is in taxoltere metro. An analog with a longer linker can easily be synthesized by incorporating a dicarboxylic acid diester instead of the carbonate between the taxane and metronidazole, e.g., 14. The *p*-nitrobenzyl ether 15 and the corresponding *p*-nitrobenzoate are radiosensitizing taxanes in which the electron-affinic group is very close to the taxane. Alternatively, hydroboration of 10 gives an alcohol, the mesylate of which reacts with, for example, 2-nitroimidazole to provide 16, and the epoxide derived by peracid treatment of 10 reacts with, for example, 2-nitroimidazole to provide 17. Ester analogs of 16 and 17 can be similarly prepared starting from 12.





It is also possible to prepare radiosensitizing
 5 taxanes having multiple radiosensitizing groups attached
 to a single linker. For example, reaction of 2-nitro
 imidazole with glycidyl chloride at somewhat elevated
 temperature provides alcohol 18, which then reacts with
 2a and with β -lactam 4 to give radiosensitizing taxane
 19.



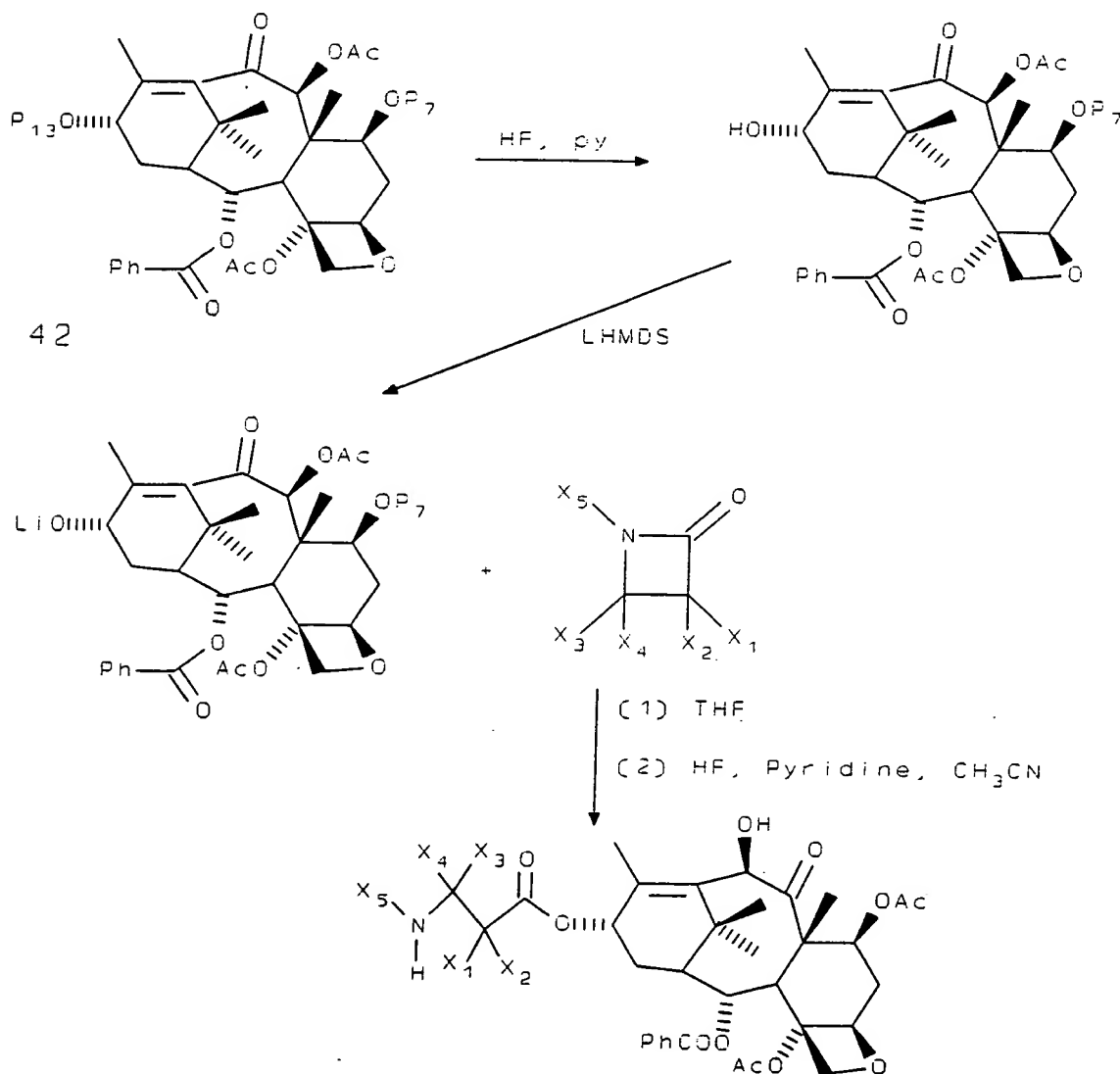
Electron-affinic moieties can be attached to the C10 position of a taxane possessing a C10 hydroxy group, such as 10-DAB, by the methods discussed for attaching the electron-affinic moieties to the C7 position. Taxanes having other C10 substituents described herein may be prepared as more fully described in PCT Patent Application WO 94/15599 and other literature references. For example, taxanes having a C10 keto substituent can be prepared by oxidation of 10-desacetyl taxanes. Taxanes which are dihydro substituted at C10 can be prepared by reacting a C10 hydroxy or acyloxy substituted taxane with samarium diiodide. Taxanes having acyloxy substituents other than acetate can be prepared by reacting the C10 hydroxy substituent of 10-deacetyl baccatin III with any standard acylating agent such as anhydrides, acid chlorides, acyl imidazoles or other activated carboxyl derivatives. Taxanes having a C10 carbonate substituent can be prepared by using an analogous chloroformate instead of the acid chloride.

Electron-affinic moieties can be attached to the C9 position of a taxane possessing a C9 hydroxy group by the methods discussed for attaching the electron-affinic moieties to the C7 position. As more fully described in PCT Patent Application WO 94/20088, the C9 the C9 keto substituent of taxol, 10-DAB, baccatin III or can be selectively reduced to yield the corresponding C9 β -hydroxy derivative with a borohydride, preferably tetrabutylammonium borohydride (Bu_4NBH_4) or triacetoxy-borohydride. The C9 β -hydroxy derivative can then be protected at C7 with a hydroxy protecting group and the C9 hydroxy group can be acylated following the methods described herein for acylation of the C7 hydroxy group.

Alternatively, reaction of 7-protected-9 β -hydroxy derivative with KH causes the acetate group (or other acyloxy group) to migrate from C10 to C9 and the hydroxy group to migrate from C9 to C10, thereby yielding a 10-desacetyl derivative, which can be acylated as described elsewhere herein.

As more fully described in PCT Patent Application WO 94/17050, C7 dihydro and other C7 substituted taxanes can be prepared by tin hydride reduction of the C7 xanthate. C7 fluoro-substituted taxanes can be prepared by treatment of C13-triethylsilyl-protected baccatin III with 2-chloro-1,1,2-trifluorotriethylamine at room temperature in THF solution. Other baccatin derivatives with a free C7 hydroxyl group behave similarly. Alternatively, 7-chloro baccatin III can be prepared by treatment of baccatin III with methanesulfonyl chloride and triethylamine in methylene chloride solution containing an excess of triethylamine hydrochloride. Taxanes having C7 acyloxy substituents can be prepared as set forth in the following reaction scheme. 7,13-protected 10-oxo-derivative is converted to its corresponding C13 alkoxide

by selectively removing the C13 protecting group and replacing it with a metal such as lithium. The alkoxide is then reacted with a β -lactam or other side chain precursor. Subsequent hydrolysis of the C7 protecting groups causes a migration of the C7 hydroxy substituent to C10, migration of the C10 oxo substituent to C9, and migration of the C9 acyloxy substituent to C7.

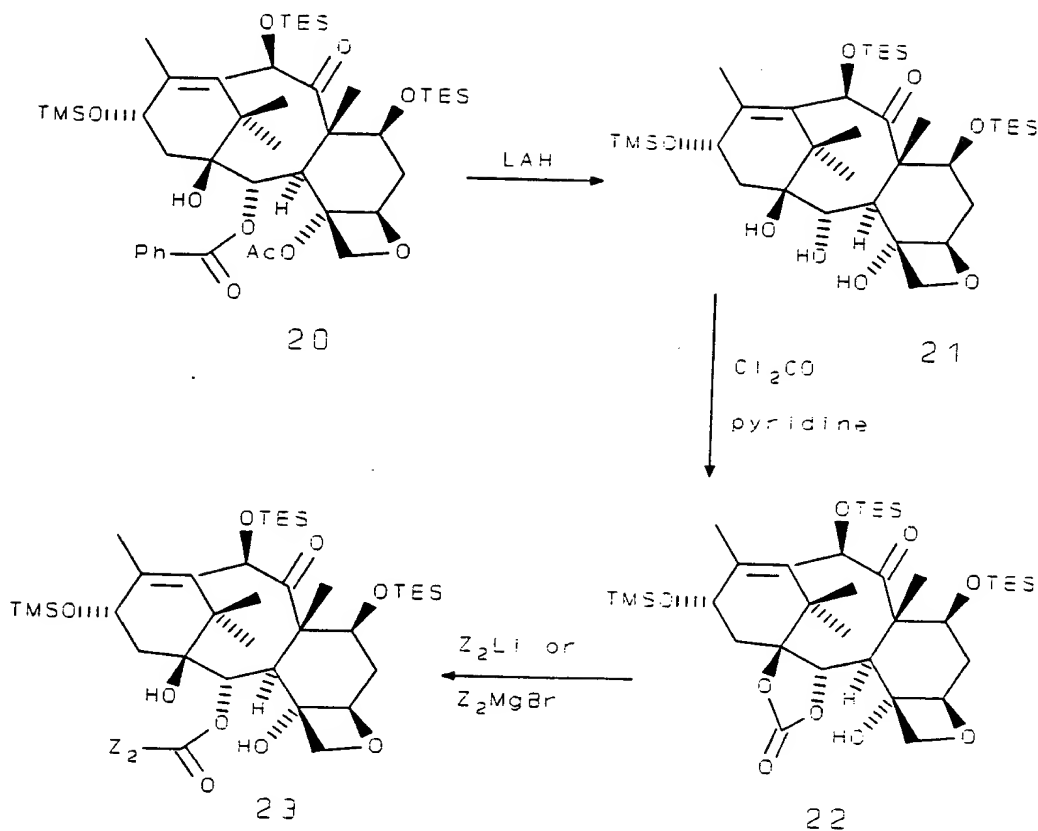


10 Taxanes having alternative C2 and/or C4 esters or carbonates which optionally may contain an electron-

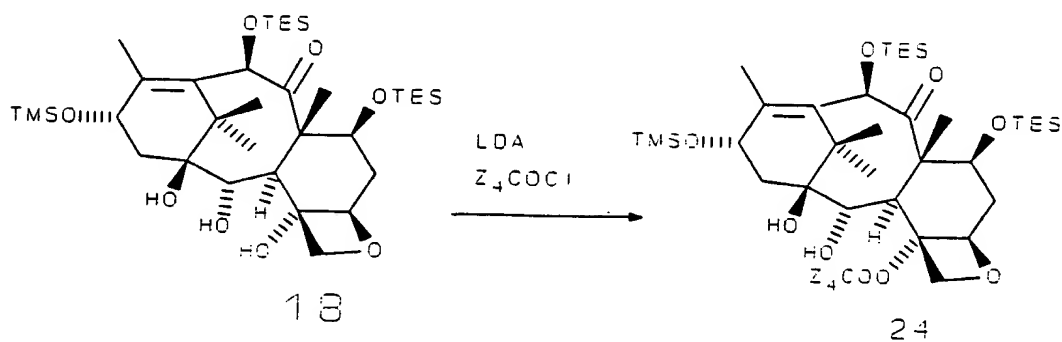
affinic moiety as described elsewhere herein can be prepared using baccatin III and 10-DAB as starting materials. The C2 and/or C4 esters of baccatin III and 10-DAB can be selectively reduced to the corresponding alcohol(s) using reducing agents such as LAH or Red-Al, and new esters can thereafter be substituted using standard acylating agents such as anhydrides and acid chlorides in combination with an amine such as pyridine, triethylamine, DMAP, or diisopropyl ethyl amine. Alternatively, the C2 and/or C4 alcohols may be converted to new C2 and/or C4 esters through formation of the corresponding alkoxide by treatment of the alcohol with a suitable base such as LDA followed by an acylating agent such as an acid chloride. The corresponding carbonates can be prepared by substituting a chloroformate for the analogous acid chloride.

Baccatin III and 10-DAB analogs having different substituents at C2 and/or C4 can be prepared as set forth in Reaction Schemes C₂-1 to C₂-5. To simplify the description, 10-DAB is used as the starting material and only the ester products are shown. It should be understood, however, that other starting materials and reactants may be substituted to yield the other C2 and C4 substituted compounds disclosed herein.

In the Reaction Scheme C₂-1, protected 10-DAB is converted to the triol 21 with lithium aluminum hydride. Triol 21 is then converted to the corresponding C4 ester using Cl₂CO in pyridine followed by a nucleophilic agent (e.g., Grignard reagents or alkylolithium reagents) wherein Z₂ is as defined elsewhere herein.

Reaction Scheme C₂-1

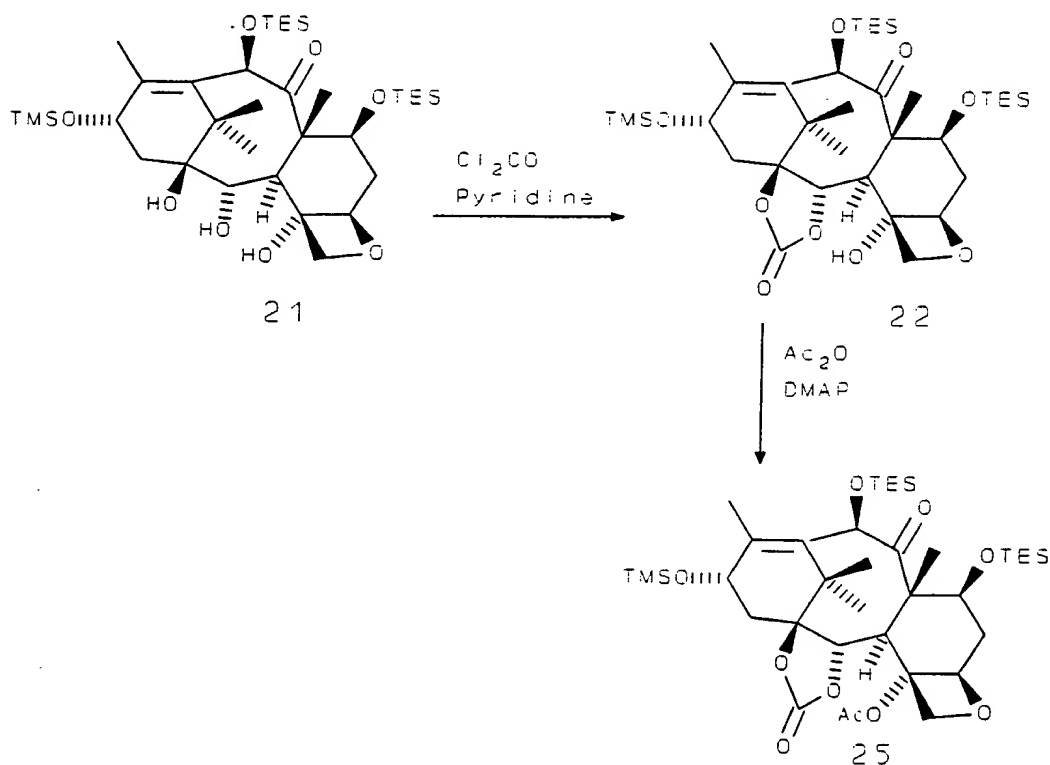
5 Deprotonation of triol 21 with LDA followed by introduction of an acid chloride selectively gives the C4 ester. For example, when acetyl chloride was used, triol 21 was converted to 1,2 diol 24 as set forth in Reaction Scheme C₂-2 wherein Z₄ is as defined elsewhere herein.

Reaction Scheme C₂-2

Triol **21** can also readily be converted to the 1,2 carbonate **22**. Acetylation of carbonate **22** under vigorous standard conditions provides carbonate **25** as described in Reaction Scheme C₂-3; addition of

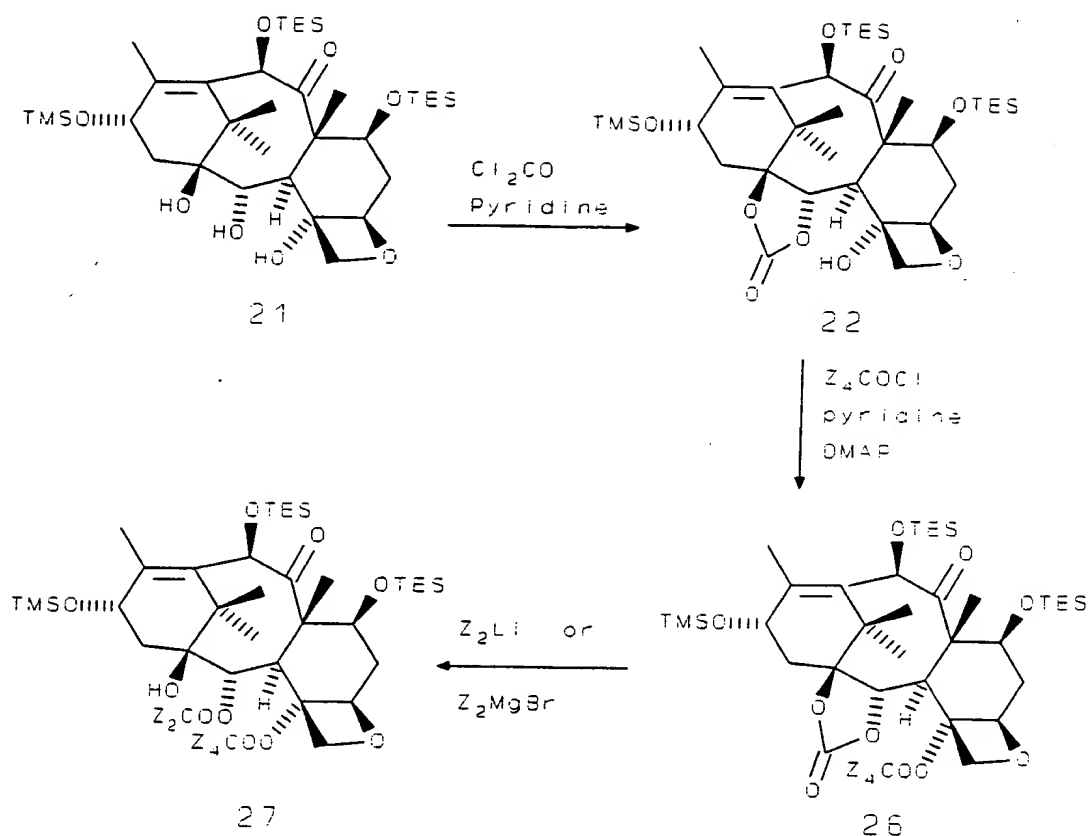
5 alkyllithiums or Grignard reagents to carbonate **22** provides the C2 ester **24** having a free hydroxyl group at C4 as set forth in Reaction Scheme C₂-1.

Reaction Scheme C₂-3

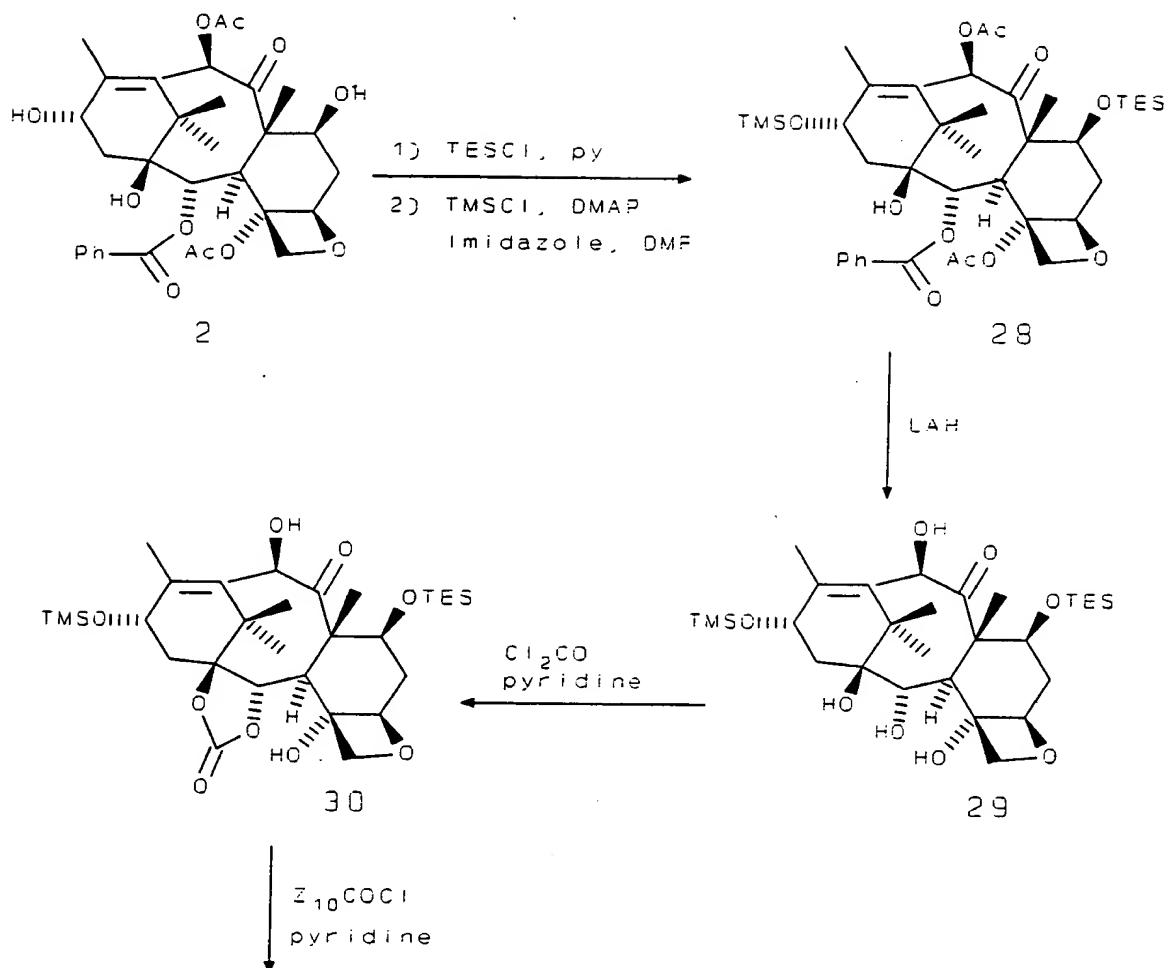


10 As set forth in Reaction Scheme C₂-4, other C4 substituents can be provided by reacting carbonate **22** with an acid chloride and a tertiary amine to yield carbonate **26** which is then reacted with alkyllithiums or Grignard reagents to provide 10-DAB derivatives **27** having

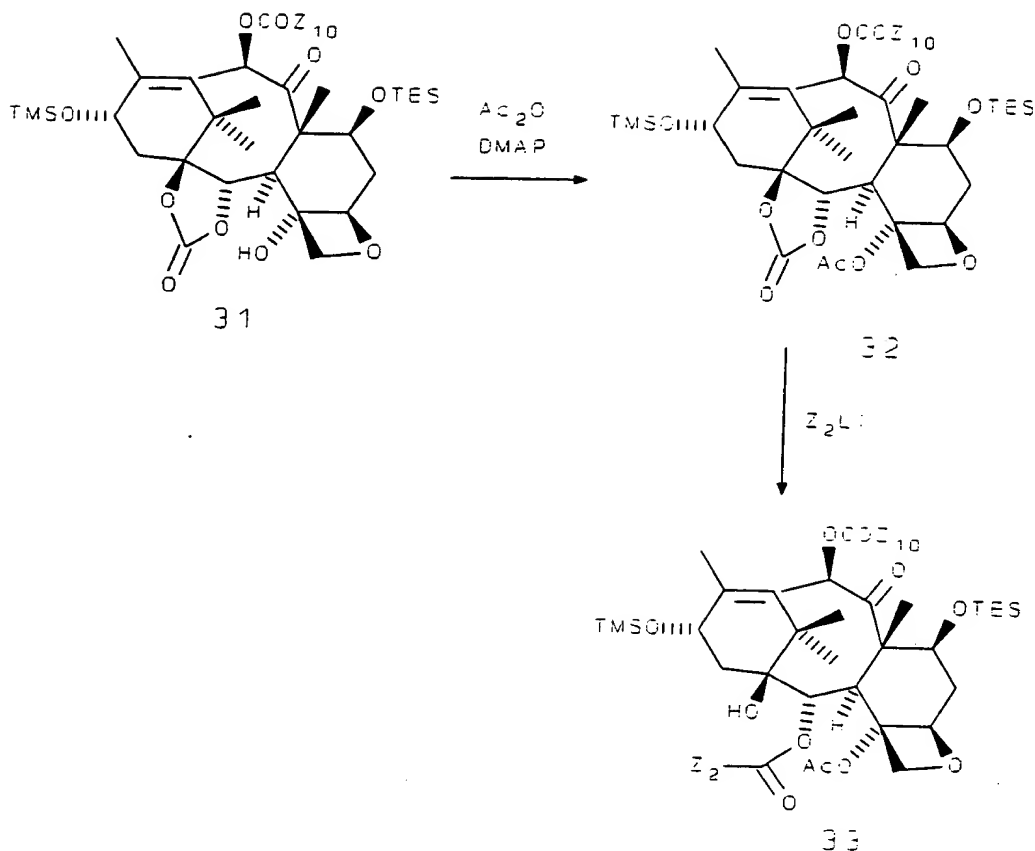
15 new substituents at C2 wherein Z₂ and Z₄ are as defined elsewhere herein.

Reaction Scheme C₂-4

Alternatively, baccatin III may be used as a starting material and reacted as shown in Reaction Scheme C₂-5. After being protected at C7 and C13, baccatin III is reduced with LAH to produce 1,2,4,10 tetraol 29. Tetraol 29 is converted to carbonate 30 using Cl_2CO and pyridine, and carbonate 30 is acylated at C10 with an acid chloride and pyridine to produce carbonate 31 (as shown) or with acetic anhydride and pyridine (not shown). Acetylation of carbonate 31 under vigorous standard conditions provides carbonate 32 which is then reacted with alkyl lithiums to provide the baccatin III derivatives 33 having new substituents at C2 and C10 wherein Z_2 and Z_{10} are as defined elsewhere herein.

Reaction Scheme C₂-5

103707-2543/660



Taxanes having radiosensitizing groups at the C14 position, i.e., the point of attachment of R₁₄, as depicted elsewhere herein, may be prepared using the same or similar methods as those described elsewhere herein with respect to attaching radiosensitizing groups to the C7 position of the taxane. The starting material for these compounds may be, for example, a hydroxylated taxane (14-hydroxy-10-deacetylbaccatin III) which has been discovered in an extract of yew needles (C&EN, p 36-37, April 12, 1993). Derivatives of this hydroxylated taxane having the various C2, C4, C7, C9, C10, C3' and C5' functional groups described above may also be prepared by using this hydroxylated taxane. In addition, the C14 hydroxy group together with the C1 hydroxy group of 10-DAB can be converted to a 1,2-carbonate as described in C&EN or it may be converted to a variety of

esters or other functional groups as otherwise described herein in connection with the C2, C4, C9 and C10 substituents.

5 The taxane radiosensitizers of the present invention can be combined with various excipient vehicles and/or adjuvants well known in this art which serve as pharmaceutically acceptable carriers to permit drug administration in the form of, e.g., injections, suspensions, emulsions, tablets, capsules, and ointments. 10 These pharmaceutical compositions, containing a radiosensitizing amount of the described substituted diamine compounds, may be administered by any acceptable means which results in the radiosensitization of tumor cells. For warm-blooded animals, and in particular, for 15 humans undergoing radiotherapy treatment, administration can be oral, parenteral, subcutaneous, intravenous, intramuscular and/or intraperitoneal. To destroy tumor cells, the pharmaceutical composition containing the radiosensitizing diamines are administered in an amount 20 effective to radiosensitize the tumor cells (in the range of 1 to 100 mg/kg for humans). The specific dosage administered will be dependent upon such factors as the general health and physical condition of the patient as well as his age and weight, the stage of the patient's 25 disease condition, and the existence of any concurrent treatments.

After administration of the radiosensitizing composition to the tumor cells and the passage of a time interval sufficient to enhance radiosensitization of the 30 tumor cells, the tumor cells are irradiated with a dose of radiation effective to destroy the tumor cells. Generally, the patient will receive a total radiation dosage of about 60 to 76 Gy over seven to eight weeks, each individual radiation dose to be given within 35 approximately 1 to 4 hours after administration of the

radiosensitizer. Such sequences of radiosensitization treatments and irradiation are repeated as needed to abate and, optimally, reduce or eliminate, the spread of the malignancy.

5

Definitions

The "hydrocarbon" moieties described herein are organic compounds or radicals consisting exclusively of the elements carbon and hydrogen. These moieties include alkyl, alkenyl, alkynyl, and aryl moieties. These moieties also include alkyl, alkenyl, alkynyl, and aryl moieties substituted with other aliphatic or cyclic hydrocarbon groups, such as alkaryl, alkenaryl and alkynaryl. Preferably, these moieties comprise 1 to 20 carbon atoms.

15

The alkyl groups described herein are preferably lower alkyl containing from one to six carbon atoms in the principal chain and up to 20 carbon atoms. They may be straight or branched chain and include methyl, ethyl, propyl, isopropyl, butyl, hexyl and the like. They may be substituted with aliphatic or cyclic hydrocarbon radicals or hetero-substituted with the various substituents defined herein.

20

The alkenyl groups described herein are preferably lower alkenyl containing from two to six carbon atoms in the principal chain and up to 20 carbon atoms. They may be straight or branched chain and include ethenyl, propenyl, isopropenyl, butenyl, isobutenyl, hexenyl, and the like. They may be substituted with aliphatic or cyclic hydrocarbon radicals or hetero-substituted with the various substituents defined herein.

25

30

The alkynyl groups described herein are preferably lower alkynyl containing from two to six carbon atoms in the principal chain and up to 20 carbon

atoms. They may be straight or branched chain and include ethynyl, propynyl, butynyl, isobutynyl, hexynyl, and the like. They may be substituted with aliphatic or cyclic hydrocarbon radicals or hetero-substituted with the various substituents defined herein.

The aryl moieties described herein contain from 6 to 20 carbon atoms and include phenyl. They may be hydrocarbon or heterosubstituted with the various substituents defined herein. Phenyl is the more preferred aryl.

The heteroaryl moieties described are heterocyclic compounds or radicals which are analogous to aromatic compounds or radicals and which contain a total of 5 to 20 atoms, usually 5 or 6 ring atoms, and at least one atom other than carbon, such as furyl, thienyl, pyridyl and the like. The heteroaryl moieties may be substituted with hydrocarbon, heterosubstituted hydrocarbon or hetero-atom containing substituents with the hetero-atoms being selected from the group consisting of nitrogen, oxygen, silicon, phosphorous, boron, sulfur, and halogens. These substituents include lower alkoxy such as methoxy, ethoxy, butoxy; halogen such as chloro or fluoro; ethers; acetals; ketals; esters; heteroaryl such as furyl or thienyl; alkanoxy; hydroxy; protected hydroxy; acyl; acyloxy; nitro; amino; and amido.

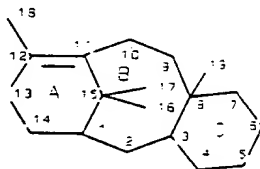
The heterosubstituted hydrocarbon moieties described herein are hydrocarbon moieties which are substituted with at least one atom other than carbon, including moieties in which a carbon chain atom is substituted with a hetero atom such as nitrogen, oxygen, silicon, phosphorous, boron, sulfur, or a halogen atom. These substituents include lower alkoxy such as methoxy, ethoxy, butoxy; halogen such as chloro or fluoro; ethers; acetals; ketals; esters; heteroaryl such as furyl or

thienyl; alkanoxy; hydroxy; protected hydroxy; acyl; acyloxy; nitro; amino; and amido.

The acyl moieties described herein contain hydrocarbon, substituted hydrocarbon or heteroaryl moieties.

The alkoxycarbonyloxy moieties described herein comprise lower hydrocarbon or substituted hydrocarbon moieties.

The term "taxane" as used herein, denotes compounds containing the A, B and C rings (with numbering of the ring positions shown herein):



As used herein "Ac" means acetyl; "AIBN" means azo-(bis)-isobutyronitrile; "Ar" means aryl; "BMDA" means BrMgNiPr₂; "BOC" means butyloxycarbonyl; "BOM" means benzyloxymethyl; "10-DAB" means 10-desacetylbaicatin III; "DBU" means diazabicycloundecane; "DMAP" means p-dimethylamino pyridine; "DDQ" means dicyano-dichloroquinone; "DMF" means dimethylformamide; "Et" means ethyl; "FAR" means 2-chloro-1,1,2-trifluoro-triethylamine; "iPr" means isopropyl; "LAH" means lithium aluminum hydride; "LDA" means lithium diisopropylamide; "LHMDS" means lithium hexamethyldisilazide; "LTMP" means lithium tetramethylpiperidide; "mCPBA" means metachloro-perbenzoic acid; "Me" means methyl; "MOP" means 2-methoxy-2-propyl; "Ms" means CH₃SO₂-; "Ph" means phenyl; "protected hydroxy" means -OP or -OT wherein P or T is a hydroxy protecting group; "py" means pyridine; "R" means lower alkyl unless otherwise defined; "Red-Al" means sodium bis(2-methoxyethoxy) aluminum hydride; "Swern"

means $(\text{COCl})_2$, Et_3N ; "TASF" means tris(diethylamino)-
sulfoniumdifluorotrimethyl-silicate; "TBAF" means
tetrabutylammonium fluoride; "tBu" and "t-Bu" means tert-
butyl; "TBS" means $\text{Me}_2\text{t-BuSi-}$; "TES" means triethylsilyl;
5 "Tf" means $-\text{SO}_2\text{CF}_3$; "TMS" means trimethyl-silyl; "TPAP"
means tetrapropylammonium perruthenate; and "Ts" means
toluenesulfonyl. "Hydroxy protecting group" includes,
but is not limited to, acetals having two to ten carbons,
ketals having two to ten carbons, ethers such as methyl,
10 t-butyl, benzyl, p-methoxybenzyl, p-nitrobenzyl, allyl,
trityl, methoxymethyl, methoxyethoxymethyl, ethoxyethyl,
tetrahydropyranyl, tetrahydrothiopyranyl, and
trialkylsilyl ethers such as trimethylsilyl ether,
triethylsilyl ether, dimethylarylsilyl ether,
15 triisopropylsilyl ether and t-butyl dimethylsilyl ether;
esters such as benzoyl, acetyl, phenylacetyl, formyl,
mono-, di-, and trihaloacetyl such as chloroacetyl,
dichloroacetyl, trichloroacetyl, trifluoroacetyl; and
carbonates including but not limited to alkyl carbonates
20 having from one to six carbon atoms such as methyl,
ethyl, n-propyl, isopropyl, n-butyl, t-butyl; isobutyl,
and n-pentyl; alkyl carbonates having from one to six
carbon atoms and substituted with one or more halogen
atoms such as 2,2,2-trichloroethoxymethyl and 2,2,2-tri-
25 chloroethyl; alkenyl carbonates having from two to six
carbon atoms such as vinyl and allyl; cycloalkyl
carbonates having from three to six carbon atoms such as
cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl; and
phenyl or benzyl carbonates optionally substituted on the
30 ring with one or more C_{1-6} alkoxy, or nitro. Other
hydroxyl protecting groups may be found in "Protective
Groups in Organic Synthesis" by T. W. Greene, John Wiley
and Sons, 1981.

To further illustrate and explain the
35 invention, several examples are presented below.

FSU 10209 PATENT

EXAMPLE 1

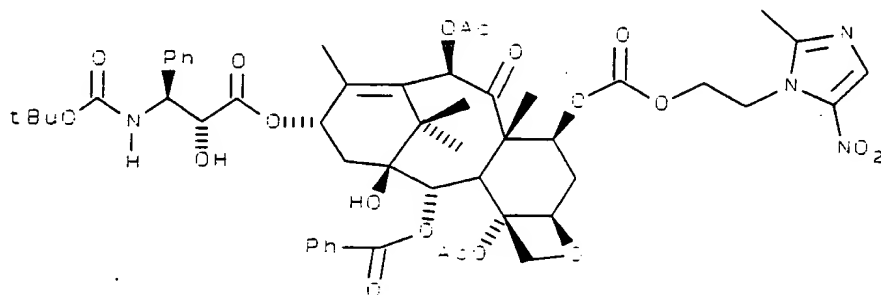
Preparation of 7-(metronidazoleoxycarbonyl)baccatin III
hn-4-99-4d-2-1

To a solution of baccatin III (100 mg, 0.170
5 mmol) in anhydrous 1,2-dichloroethane (1 mL) under
nitrogen was added 1,1'-carbonyldiimidazole (55 mg, 0.40
mmol) and the reaction mixture was warmed up to 60 °C and
stirred at that temperature for 10 h at which time the
reaction was complete. Metronidazole (292 mg, 1.72 mmol)
10 was added (neat) and the mixture was refluxed under
nitrogen atmosphere. The progress of the reaction was
monitored by NMR. When the reaction was complete
(approximately three days) the mixture was diluted with
ethyl acetate and washed with saturated sodium
15 bicarbonate, brine. The organic layer was separated,
dried and concentrated. The crude mixture was purified
by flash chromatography to give 81 mg (70%) of the 7-
(metronidazole-oxycarbonyl)baccatin III: mp. 220-223
°C; $[\alpha]_{\text{Hg}}^{25} = -44.9$

20 ¹H NMR (CDCl₃, 500 MHz) δ 8.10 (d, J=7.1 Hz, 2H,
benzoate), 7.96 (s, 1H, imidazole), 7.62-7.46 (m, 3H,
benzoate), 6.28 (s, 1H, H10), 5.61 (d, J=6.8 Hz, 1H,
H2β), 5.49 (m, 1H, H7) 4.96 (d, J=8.2 Hz, 1H, H5), 4.85
(br s, 1H, H¹³) 4.62-4.43 (m, 4H, methylenes), 4.31 (d,
25 J=8.5 Hz, 1H, H20α), 4.13 (d, J=7.5 Hz, 1H, H20β), 3.99
(d, J=6.8 Hz, 1H, H3), 2.59 (m, 1H, H6α), 2.50 (s, 3H,
CH3-imidazole), 2.29 (m, 5H, 4Ac, H14's), 2.13 (s, 3H,
10Ac), 2.10 (br s, 3H, Me18) 2.06 (d, J=5 Hz, 1H, 13OH),
1.9 (m, 1H, H6β), 1.77 (s, 3H, Me19), 1.59 (s, 1H, 1OH),
30 1.17 (s, 3H, Me17), 1.08 (s, 3H, Me16).

EXAMPLE 2

Preparation of N-debenzoyl-N-(t-butylcarbamoyl)-
7-(metronidazoleoxycarbonyl) taxol. (Taxoltere metro)



5 hn-4-119-2

To a solution of 7-(metronidazoleoxycarbonyl) baccatin III (31 mg, 0.039 mmol) in 0.3 mL of THF at -45 °C was added dropwise 0.048 mL of a 1.00 M solution of lithium bis(trimethylsilyl)amide in THF. After 0.5 h at -45 °C, a solution of *cis*-1-(*t*-butoxycarbonyl)-3-triethylsilyloxy-4-phenylazetidin-2-one (75 mg, 0.20 mmol) in 0.3 mL of THF was added dropwise to the mixture. The solution was warmed to 0 °C and kept at that temperature for 1 h before 0.2 mL of a 10% solution of AcOH in THF was added. The mixture was partitioned between saturated aqueous NaHCO₃ and 60/40 ethyl acetate/hexane. Evaporation of the organic layer gave a residue which was purified by filtration through silica gel to give 45.0 mg of a mixture containing (2'*R*,3'*S*)-2'-triethylsilyl-*N*-debenzoyl-*N*-(*t*-butylcarbamoyl)-7-(metronidazoleoxycarbonyl) taxol and a small amount of the (2'*S*,3'*R*) isomer.

To a solution of 45.0 mg of the mixture obtained from the previous reaction in 1.5 mL of acetonitrile and 0.6 mL of pyridine at 0 °C was added 0.2 mL of 48% aqueous HF. The mixture was stirred at 0 °C for 3 h, then at 25 °C for 13 h, and partitioned between saturated aqueous sodium bicarbonate and ethyl acetate.

Evaporation of the ethyl acetate solution gave 40.8 mg of material which was purified by plug filtration and recrystallization from methanol/water to give 32.3 mg (79%) of N-debenzoyl-N-(t-butylcarbamoyl)-7-

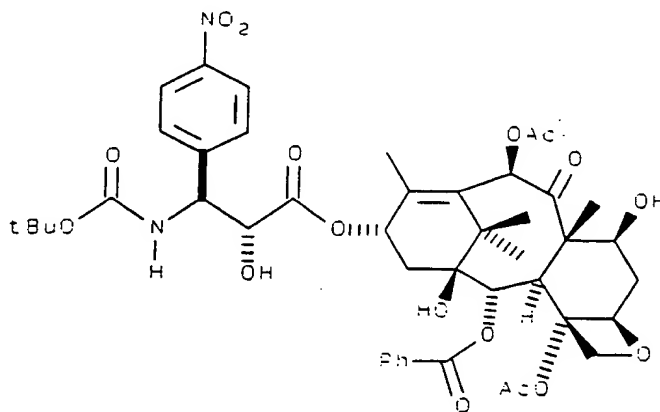
- 5 (metronidazoleoxy-carbonyl) taxol. m.p. 169-172 °C; $[\alpha]^{25}_{Na}$ -52 °C (0.0035, CHCl₃).

- ¹H NMR (CDCl₃, 300 MHz) δ 8.10 (d, J=7.1 Hz, 2H, benzoate), 7.96 (s, 1H, imidazole), 7.62-7.26 (m, 8H, benzoate, 3'-phenyl), 6.25 (s, 1H, H10), 6.18 (dd, J = 8.8, 8.8 Hz, 1H, H13), 5.65 (d, J=7.1 Hz, 1H, H2 β), 5.44-5.21 (m, 3H, NH, H3', H2'), 4.91 (d, J=9.9 Hz, 1H, H5), 4.62 (m, 4H, methylenes), 4.44 (m, 1H, H7), 4.31 (d, J = 8.2 Hz, 1H, H20 α), 4.15 (d, J = 8.2 Hz, 1H, H20 β), 3.90 (d, J=7.1 Hz, 1H, H3), 3.35 (d, J=5.5 Hz, 1H, 2'OH), 2.59 (m, 1H, H6 α), 2.49 (s, 3H, CH₃-imidazole), 2.36 (s, 3H, 4Ac), 2.31 (m, 2H, H14), 2.13 (s, 3H, 10Ac), 1.95 (m, 1H, H6 β), 1.88 (br s, 3H, Me18), 1.77 (s, 3H, Me19), 1.70 (s, 1H, 1OH), 1.34 (s, 9H, t-butyl), 1.23 (s, 3H, Me17), 1.15 (s, 3H, Me16).

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EXAMPLE 3

Preparation of N-debenzoyl-N-(t-butylcarbamoyl)-3'-desphenyl-3'-(4-nitrophenyl) taxol. (Taxoltere p-nip)



To a solution of 7-triethylsilyl baccatin III (120 mg, 0.171 mmol) in 1.2 mL of THF at -45 °C was added dropwise 0.104 mL of a 1.63 M solution of nBuLi in hexane. After 0.5 h at -45 °C, a solution of *cis*-1-(*t*-butoxycarbonyl)-3-triethylsilyloxy-4-(4-nitrophenyl)azetidin-2-one (361 mg, 0.885 mmol) in 1.2 mL of THF was added dropwise to the mixture. The solution was warmed to 0 °C and kept at that temperature for 1 h before 1 mL of a 10% solution of AcOH in THF was added. The mixture was partitioned between saturated aqueous NaHCO₃ and 60/40 ethyl acetate/hexane. Evaporation of the organic layer gave a residue which was purified by filtration through silica gel to give 192 mg of a mixture containing (2'R,3'S)-2',7-(bis)triethylsilyl-N-debenzoyl-N-(*t*-butylcarbamoyl)-3'-desphenyl-3'-(4-nitrophenyl) taxol and a very small amount of the (2'S,3'R) isomer.

To a solution of 192 mg of the mixture obtained from the previous reaction in 11 mL of acetonitrile and 0.55 mL of pyridine at 0 °C was added 1.7 mL of 48% aqueous HF. The mixture was stirred at 0 °C for 3 h, then at 25 °C for 13 h, and partitioned between saturated aqueous sodium bicarbonate and ethyl acetate. Evaporation of the ethyl acetate solution gave 153 mg of material which was purified by flash chromatography to give 140 mg (91%) of N-debenzoyl-N-(*t*-butylcarbamoyl)-3'-desphenyl-3'-(4-nitrophenyl) taxol, which was recrystallized from methanol/water. m.p. 172-173 °C; $[\alpha]^{25}_{\text{Na}}$ -54 °C (c 0.0046, CHCl₃).

EXAMPLE 4

30 Biological Studies of Taxoltere Metro and Taxoltere p-nip

4.1. In vitro chemotherapeutic activity.

Chinese hamster ovary (CHO) and human colon carcinoma (HCT-116) cells were treated with different

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concentrations of taxol, taxoltere metro, or taxoltere p-nip at 37 °C for 24 hours. Cell survival was evaluated by the colony forming assay. As shown in Figures 1 and 2, both taxoltere metro and taxoltere p-nip are much more efficient than taxol in killing CHO and HCT-116 cells. At the 50% survival level, taxoltere metro is about 15 times, and taxoltere p-nip is about 45 times, more effective than taxol in killing CHO cells; measured at the 1% survival level, taxoltere metro is about 10 times, and taxoltere p-nip is about 30 times, more effective than taxol in killing HCT-116 cells. As illustrated in Figures 1 and 2, both taxoltere metro and taxoltere p-nip exhibit significantly stronger ability than taxol to kill both types of cells at every drug dose point.

4.2. In vitro chemotherapeutic radiosensitization. These studies were carried out as above except that cells were irradiated (General Electric Maxitron 300 at 250 kvp and 20 mA (HVL 20 mm Al filter; dose rate of 2 Gy/min) after two hours incubation. Figures 3 and 4 show the results of experiments in which cells were subjected to different radiation doses in the presence or absence of drugs. Both taxoltere metro and taxoltere p-nip strongly radiosensitize both CHO and HCT-116 cells, although taxol does not. For CHO cells, the sensitizer enhancement ratio (SER) is 2.3 for 100 nM taxoltere metro and 1.6 for taxoltere p-nip. For HCT-116 cells, taxoltere metro shows a SER of 1.2 at the (low) dose of 10 nM, and taxoltere p-nip has a SER of 1.5. At each radiation dose point, there is a significantly enhanced decrease in the surviving fraction for the taxoltere metro and taxoltere p-nip treated groups, but not for the taxol treated groups. HCT-116 cells are more sensitive to both taxoltere metro and taxoltere p-nip than CHO cells, hence lower concentrations of the drugs

are required for a significant enhancement of radiation induced cell killing.

4.3. **In vivo drug toxicity.** Acute toxicity experiments were conducted on C3H/HeJ mice. The LD₅₀ (lethal dose to 50% of animals) values were determined by standard procedures described by Chan and Hayes (Chan, P.K. and Hayes, A.W. Principles and Methods for acute toxicity and eye irritancy. In Hayes, A.W., ed. Principles and Methods of Toxicity. 2nd Ed., New York, New York, Raven Press; 1989: 169220). The LD_{50/5} for i.p. taxoltere metro is 249.67 mg/kg, compared with 140.97 mg/kg for i.p. taxol. At high drug dose levels, the death of mice in the taxol treated groups occurred sooner than the death of mice in the taxoltere metro treated groups. Severe toxic symptoms such as the constriction of pupils and the contraction of erectile tissue of hair follicles (resulting in rough hair) were observed 24 hours after drug administration in the taxol treated groups, but not in the taxoltere metro treated groups. Obviously, the acute toxicity of taxoltere metro is significantly lower than that of taxol. The LD_{50/5} values are 79.13 mg/kg for i.p. administration of taxoltere p-nip and 134.16 mg/kg for i.v. administration of taxoltere p-nip. The data strongly demonstrate that taxoltere p-nip is significantly less toxic when administered i.v. than it is when administered i.p.

4.4. **In vivo chemotherapeutic activity.** Taxol, taxoltere metro, and taxoltere p-nip were administered at equitoxic doses using C3H/HeJ mice bearing a mouse mammary adenocarcinoma (MTG-B) in the right flank. As illustrated in Figure 5, taxol-metro is much more effective than taxol in increasing the life span of mammary tumor-bearing mice. The survival time

for control mice was 13 days. At 40% of the LD_{50/5} dose, taxol increased the survival time by 31% to 17 days, and taxoltere metro increased the survival time by 123% to 29 days. Taxoltere p-nip (Figure 6) is substantially more potent, increasing the life span by 1015% to 145 days (dose-response data not shown because only a single dose (40% of LD_{50/5}) was studied). Thus, under equitoxic conditions, taxoltere metro was 4 times, and taxoltere p-nip was 33 times, more effective than taxol.

Two experiments in which fractionated lower doses (20% of LD_{50/5}) of taxol, taxoltere metro and taxoltere p-nip were administered to MTG-B tumor bearing C3H/Hej mice have been carried out. In the first of these, drugs were administered on a q7d x 4 schedule (Figure 7). As shown in Figure 8, tumor doubling times are greatly extended by taxoltere metro and are even further extended by taxoltere p-nip on this administration schedule. In the second experiment (Figure 9), drugs were administered on a q11d x4 schedule. Using this schedule, tumor doubling times (shown in Figure 10) are again greatly extended, with taxoltere metro producing the better results. Efficacy is positively correlated with frequency of injection (i.e., smaller intervals between injections). On both schedules, taxoltere metro and taxoltere p-nip exhibit significantly stronger antitumor effects than taxol.

4.5. In vivo chemotherapeutic radiosensitization. MTG-B tumor bearing C3H/Hej mice received single equitoxic drug doses i.p. (40% of LD_{50/5}). One day later the mice were placed in a lead holder (with the tumor-bearing hind leg exposed), and the tumor was subjected to a 22 Gy Xray dose (Figure 11). As shown in Figure 12, tumor doubling times (TDT) of 3.5 days (control), 8.5 days (22 Gy Xray exposure alone), 14.5

days (taxol alone), 19.5 days (taxol plus 22 Gy Xray), 20.5 days (taxoltere metro alone), and 29.5 days (taxoltere metro plus 22 Gy Xray) were observed. Equally large effects were observed when mice were given

5 taxoltere metro two hours prior to irradiation (data not shown). Taxoltere p-nip alone (TDT 3D 36.5 days) is even more effective in delaying tumor growth (Figure 12), and the combination of taxoltere p-nip plus 22 Gy produced a TDT of 65 days.

10 Administration of taxoltere p-nip i.v. (24% of $LD_{50/5}$) (Figure 13), gave a tumor doubling time of 52 days (Figure 14), approximately equivalent to the results obtained from i.p. administration.

4.6. Cure Rates. The term "cure" is defined
15 by the U.S. National Cancer Institute as tumor-free survival for at least twice the survival time of control tumor-bearing mice, therefore we have used 28 days tumor-free to define a "cure" in this system. The cure rate is 40% for mice treated i.p. with taxoltere p-nip alone at a single dose, and 75% for the combination of
20 taxoltere p-nip and 22 Gy (Figure 15). Similarly, a single i.v. injection of taxoltere p-nip alone induces a cure rate of 17%, and an 83% cure rate was observed for the combination of taxoltere p-nip and 22 Gy (Figure 16),
25 even though this experiment was conducted with a lower dose (24% of $LD_{50/5}$). Although in this latter group one tumor recurred after 40 days, some treated mice have remained tumor-free for more than one year, and still survive.

30 As shown in Figure 17, administration of taxoltere p-nip iv at 40% of $LD_{50/5}$ produced a cure rate of 50%, and iv administration of taxoltere p-nip at 40% of $LD_{50/5}$ in combination with 22 Gy radiation produced a cure

rate of 100%. Cure rate as a function of drug dose is shown in Figure 18.

In view of the above, it will be seen that the several objects of the invention are achieved and other
5 advantageous results attained.

As various changes could be made in the above compounds and methods without departing from the scope of the invention it is intended that all matter contained in the above description shall be interpreted as
10 illustrative and not in a limiting sense.

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